DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application]This invention relates to the method of producing saponin which is the drug effect main ingredients of a medicinal ginseng with high yield, about the culturing method of the callus which makes a medicinal ginseng origin vegetation.
[0002]
[Description of the Prior Art]That by which the medicinal ginseng is generally used widely although various kinds, such as the Kuangtung ginseng, a bamboo joint ginseng, and 37 ginsengs, are known is another-name Panax schinseng (Panax ginseng C.A.Meyer) called "ginseng." A medicinal ginseng is an outstanding Chinese medicine which has drug effect, such as sthenia, strong energy, hemopoiesis, a stillness operation, etc. besides the central inhibition and an anti-fatigue operation.
[0003]The subject of the medicinal properties is saponin and sapogenin of triterpenoid which are called the JINSENO side (ginsenoside). Although the saponin extracted from a medicinal ginseng contains many the component groups Ro, Ra, and Rb, Rc, Rd, Re, Rf, Rg(s), and Rh(s), it is Rb and Rg which make the center of drug effect. Rb group acts in [Rg group] agitation restrainedly to a central nervous system.
[0004]Now, as for a ginseng, the thing of wild nature hardly exists, but the supply is made mainly by cultivation. Harvest takes in several years and it is weak also to a damage by blight and harmful insects, once it moreover grows [disagreeable] sequential cropping to a degree very much, 20 years or more cannot be planted a field with the same crop every year, and productivity of a ginseng is remarkably low. The good land of wastewater of cool high ground is chosen as the cultivation hot summer also. There is also a problem that a saponin content, an ingredient ratio, etc. are not fixed by origin, extraction stage, cultivation years, etc. in a cultivation article further again.
[0005]Then, research for supplying a medicinal ginseng, without receiving influence in the weather and other natural conditions with tissue culture method is done early, It is shown clearly that the saponin extracted from the callus produced by cultivating the raw organization of a medicinal ginseng has same natural medicinal ginseng, extraction ingredient, and drug effect that were obtained by cultivation (JP,48-31917,B, JP,63-21470,B, JP,63-21471,B, etc.).
[0006]In order to make the yield of a medicinal ginseng tissue culture thing increase, the method of only adding nutrients, such as a casein digest, quantitatively to the culture medium for plant tissue culture is performed, but it not only may make the presentation of a culture medium complicate, but in culture of a plant tissue, this may cause a growth obstacle. And even if the yield of a ginseng callus increases, the saponin content per cell may fall.
[0007]In order to make the yield of saponin per cell increase, the method of adding the intermediate of saponin biosyntheses, such as mevalonic acid, to the culture medium for cell cultures is also performed, but. Since the effect changes with the kinds, the concentration, and the stages to add of a biosynthetic intermediate, it will complicate not only to a medium composition but to a culturing method.
[0008]
[Problem(s) to be Solved by the Invention]Being under the above situations, this invention aims simply and effective at offer of the culturing method of the medicinal ginseng callus which can produce saponin. This invention aims at offer of the culturing method of the medicinal ginseng callus to which

the quantity of production of saponin is made to increase, without making the presentation of a culture medium complicate.

[0009]

[Means for Solving the Problem]Face this invention cultivating a callus which is a formless cell lump made by cutting some medicinal ginsengs (Panax ginseng C.A.Meyer) generally used for a raw material of Chinese orthodox medicine, etc., and cultivating and carrying out fissiparity under suitable conditions, and it is performed under a specific culture condition, It is the method of producing saponin which is the drug effect main ingredients of a medicinal ginseng with high yield.

[0010]As for manufacture of saponin using a cultured cell of a medicinal ginseng, what increases a callus as a culture medium and promotes production of saponin which is secondary metabolite is desirable. this invention person performs search of a kind and combination of a vegetable growth regulator which was suitable for production of saponin which is growth and secondary metabolite of a callus first, An effect is high when a culture medium which contains naphthaleneacetic acid (it may be hereafter called "NAA" for short.) in callus proliferation carries out NAA independent addition, When a culture medium which contains gibberellin (it may be hereafter called "GA" for short.) in saponin formation carries out GA independent addition, an effect finds out a high thing, it inquired wholeheartedly based on the discovery, and it was made to complete this invention.

[0011]This invention is a culturing method of a medicinal ginseng callus carrying out the passage of the callus which makes a medicinal ginseng origin vegetation to a culture medium of different conditions.

[0012]It becomes a culture medium of conditions different [account of the upper] from combination of a culture medium which promotes production of a culture medium and saponin which were preferably suitable for growth of a callus. Therefore, this invention is a subculture method of a medicinal ginseng callus which uses combination of a culture medium which promotes production of a culture medium and saponin suitable for growth of a callus.

[0013]. These culture media are basal media for plant tissue culture currently used from the former. For example, a culture medium which made a basal medium MS liquid medium (Murasige and Skoog Mr. culture medium), and was suitable for growth of a callus at the basal medium, It is the culture medium which added naphthaleneacetic acid as a plant growth-regulating substance, and a culture medium which promotes production of saponin is a culture medium which added gibberellin as a plant growth-regulating substance.

[0014]In this invention, a culture medium of another side is used for two kinds of culture media of different conditions as a secondary culture medium by making one culture medium into a primary culture medium. That is, when using a culture medium suitable for growth of a callus as a primary culture medium, a culture medium which promotes production of saponin is used as a secondary culture medium. When using a culture medium which promotes production of saponin as a primary culture medium, a culture medium suitable for growth of a callus is used as a secondary culture medium.

[0015]Although this invention performs two-step cultivation which carries out subculture combining a culture medium of different conditions as above-mentioned, especially as long as what generation is cultivated in each stage has good production of saponin, there is no restriction. A mode of two generation cultivated a total of three generation is illustrated as a desirable mode by one generation and a secondary culture medium by a primary culture medium.

[0016]Hereafter, a mode use a NAA culture medium as a primary culture medium, and using GA culture medium as a secondary culture medium is explained. After cultivating a callus which makes a medicinal

ginseng origin vegetation by a NAA culture medium, the passage of it is carried out to GA culture medium. In order to raise the quantity of production of saponin more, after cultivating by a NAA culture medium and carrying out a passage to GA culture medium as an effective mode, a passage is further carried out to GA culture medium. Especially as long as what generation is cultivated by NAA culture medium or GA culture medium has good production of saponin, there is no restriction, but a mode of two generation cultivated a total of three generation is preferred at one generation and GA culture medium in a NAA culture medium, in view of the productivity of saponin, and the rationality of incubation period.

[0017]On the occasion of transplantation to a secondary culture medium from a primary culture medium, not to mention NAA in a primary culture medium has an adverse effect on a secondary culture medium, it has had good influence. If the passage of the callus is carried out to GA culture medium from a NAA culture medium, a breeding ratio should fall, because the NAA culture medium of a breeding ratio is better than GA culture medium when a callus is cultivated by each culture medium. However, actually, even if it carries out a passage to GA culture medium from a NAA culture medium, a breeding ratio does not fall. As the reason, character in which the physiological functions (extension of a cell, promotion of division, etc.) are especially effective under existence (for example, NAA, 2, 4-D, IBA, etc.) of auxin is mentioned to GA. So, it seems that a synergistic effect is brought to increase of production of saponin.

[0018]

[Function]The quantity of production of saponin can be raised by performing two-step culture as mentioned above. That is, saponin which is the same medicinal properties as a natural medicinal ginseng is producible with high yield by performing the method of carrying out a passage to the culture medium from which conditions differ, and cultivating, i.e., two-step culture, rather than cultivating a medicinal ginseng callus by the culture medium of the same conditions like before. If one more generation is cultivated further and a total of three generation is cultivated by a secondary culture medium in two-step cultivation, the quantity of production of saponin can be raised remarkably.

[0019]

[Example]An example explains the details of the invention in this application. The invention in this application is not limited at all by these examples. The medicinal ginseng callus used for the example below rinses a fourth grader ginseng, The explant cut abacterially is made into a growth regulator after surface sterilization, and it is acetic acid (2, 4-D). It ****ed to the agar MS culture medium which added 1 ppm and kinetin (Kinetin) 0.1ppm, and callus induction was carried out at 25 ** under the dark place. The derived calluses are 1.0 ppm indolebutyric acid (IBA) and kinetin. The passage was carried out by the agar MS culture medium which added 0.1 ppm, and acetic acid was removed. What carried out preculture by the same culture medium as a primary culture medium beforehand was used for the callus with which an experiment is presented in order to lose the influence of the growth regulator of a passage culture medium.

[0020]The example 1MS liquid medium was made into the basal medium, what carried out NAA2ppm addition as a vegetable growth regulator was made into the primary culture medium, and what carried out GA2ppm addition was made into the secondary culture medium. 30 ml of the above-mentioned culture media were put into a 100-ml Erlenmeyer flask, and autoclaving was carried out to it. A 2-g medicinal ginseng callus was transplanted to this, and the rotary shaker of 80r.p.m performed dark place culture for three weeks at 25 **. After culture, it freeze-dried, after demineralized water washed, and the

callus made the weight dry weight.
[0021]The extraction method of saponin is as follows. The mortar ground the freeze-dried callus, it added 50 ml of methanol, and digested it at 40-50 ** for 3 hours. This was repeated twice and the methanolic extract was obtained. This was dissolved in 50 ml of demineralized water, it put into the separating funnel, and 50 ml of ether washed twice. 50 ml of water saturation n-butanol extracted the water layer (lower layer) twice with n-butanol saturated water further, and the water saturation n-butanol layer (upper layer) was obtained. Rough saponin was obtained for this solvent distilling out and by carrying out reduced pressure drying.
[0022]the preparation of this rough saponin, natural saponin and Ginsenoside-Rb_1, and Ginsenoside-Rg_1 -- thin layer chromatography -- having developed (n-butanol: developing solvent : ethyl acetate : water =4:5:1) -- time -- this rough saponin -- an Rf value. The pattern was completely in agreement with the natural article.
[0023]The measuring curve was created by the high-speed thin layer chromatoscanner, and the content of a Ginsenoside-Rb group and a Ginsenoside-Rg group was calculated. And each group's sum total was made into the total amount of saponin. The amount of saponin per dry weight was calculated from this value.
[0024]The primary culture medium compared one generation with what was cultivated two generation by the primary culture medium, and the secondary culture medium compared the thing of one generation cultivated a total of two generation. The result of culture was shown in drawing 1. In the total amount of saponin, 1.3 times and the amount of saponin per dry weight increased receipts 1.5 times by transplanting to a secondary culture medium from a primary culture medium.
[0025]The culture condition of the kind and concentration of example 2 culture medium and a vegetable growth regulator, and others was made the same as that of Example 1. And the primary culture medium compared one generation with what was cultivated three generation by the primary culture medium, and the secondary culture medium compared the thing of two generation cultivated a total of three generation. The result was shown in drawing 2. In the total amount of saponin, 1.5 times and the amount of saponin per dry weight increased receipts 1.7 times by cultivating two generation by one generation and a secondary culture medium by a primary culture medium.
[0026]The result of having contrasted Example 1 and Example 2 was shown in drawing 3. The increase-of-income effect of saponin was seen more for the method of cultivating two generation rather than having cultivated one generation by a secondary culture medium after culture by a primary culture medium.
[0027]The example 3MS liquid medium was made into the basal medium, what carried out NAA5ppm addition as a plant growth-regulating substance was made into the primary culture medium, what carried out GA0.001ppm addition was made into the secondary culture medium, the primary culture medium compared one generation with what was cultivated three generation by the primary culture medium, and the secondary culture medium compared the thing of two generation cultivated a total of three generation. The result was shown in drawing 4. In the total amount of saponin, 3.7 times and saponin per dry weight increased receipts 2.3 times by cultivating two generation by one generation and a secondary culture medium by a primary culture medium.
[0028]The example 4MS liquid medium was made into the basal medium, what carried out GA2ppm addition as a plant growth-regulating substance was made into the primary culture medium, what carried

out NAA2ppm addition was made into the secondary culture medium, the primary culture medium compared one generation with what was cultivated two generation by the primary culture medium, and the secondary culture medium compared the thing of one generation cultivated a total of two generation. A result is shown in drawing 5. In the total amount of saponin, from the primary culture medium, 1.6 times and the amount of saponin per dry weight increased receipts 1.9 times by transplanting to a secondary culture medium.

[0029]The example 5MS liquid medium was made into the basal medium, what added a primary culture medium and 5 ppm of NAA(s) for what added 2 ppm of GA(s) as a plant growth-regulating substance was made into the secondary culture medium, the primary culture medium compared one generation with what was cultivated three generation by the primary culture medium, and the secondary culture medium compared the thing of two generation cultivated a total of three generation. A result is shown in drawing 6. In the total amount of saponin, 3.0 times and the amount of saponin per dry weight increased receipts 2.7 times by cultivating two generation by one generation and a secondary culture medium by a primary culture medium.

[0030]

[Effect of the Invention]The culturing method of the medicinal ginseng callus which can produce saponin can be provided simply and effectively. The culturing method of the medicinal ginseng callus to which the quantity of production of saponin is made to increase can be provided without making the presentation of a culture medium complicate.